Kidney International, Vol. 45 (1994), pp. 1211–1214

TECHNICAL NOTE

Reliable atomic absorption analysis of sodium and potassium in rat renal tubular fluid

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The technique of graphite furnace atomic absorption spectrometry for analysis of sodium and potassium in tubular fluid samples is commonly used [1–3]. In the atomic state, sodium as well as potassium absorb light at three wavelengths, which have different sensitivities when they are used for analysis of the elements. The relative sensitivities of these absorption wavelenghts are shown in Table 1.

Generally, sodium is measured at one of the two most sensitive wavelengths: 589.0 nm [1, 2] or 589.6 nm [3]. In the literature, analysis of 0.1 to 0.2 nl samples has been reported after deposition of the sample with a small constriction pipette and a micromanipulator on a removable graphite microboat, which fits in a rectangular graphite cuvette [1, 2]. Analysis of larger volumes (20 nl) after a 20,000-fold dilution step with ultrapure water and manually pipetting into a cylindrical cuvette through an opening in the wall has also been described [3]. Measurement by autosampler at an insensitive wavelength (330.2 nm) after 1,000-fold dilution has been performed [4], but this requires very large samples (200 nl). Potassium is usually measured at 766.5 nm, the most sensitive wavelength [1–3]. Concentrations of sodium in both proximal and distal tubular fluid are usually about 30 × higher than those of potassium, whereas the sensitivity for potassium is equal to or only slightly higher than the sensitivity for sodium. By consequence, simultaneous measurement of sodium and potassium is hampered by the choice between lack of linearity for sodium and lack of sensitivity for potassium [2, 3]. Because of the extremely high sensitivity at the most sensitive wavelengths, the determination of sodium is notoriously difficult, because contamination by sodium from air dust [2] or water used as diluent [3] occurs frequently.

We developed a method for simultaneous measurement of sodium and potassium in 2 nl samples with the aims to minimize the relative contribution to the absorption signal of sodium from dust, and to bring the potassium-to-sodium absorbance ratio into the same range as the sodium-to-potassium concentration ratio. This was achieved by measuring the elements at less sensitive wavelenghts (330.2 and 769.9 nm, respectively), at which the sensitivity for sodium is reduced notably more than for potassium.

Received for publication September 20, 1993
and in revised form November 22, 1993
Accepted for publication November 22, 1993

Methods

Reagents

To assess the effect of the presence of other solutes, standards were dissolved in ultrapure water (UPW) and artificial tubular fluid (ATF). Standards in UPW (Millipore Alpha-Q system, Etten-Leur, The Netherlands) contained 40, 80, 120, and 160 mmol/liter NaCl or 2, 4, 6, and 8 mmol/liter KCl. A solution containing $NaHCO_3$, 10 mmol/liter; NaH_2PO_4, 1 mmol/liter; $MgSO_4$ 0.5 mmol/liter; $CaCl_2$, 0.5 mmol/liter; urea, 5 mmol/liter; glucose, 2.5 mmol/liter; inulin, 2 mg/l was made for constitution of standards in ATF and quality control solutions. Sodium standards in ATF comprised 5 mmol/liter KCl, and 29, 69, 109, or 149 mmol/liter NaCl in this solution, potassium standards 149 mmol/liter NaCl, and 2, 4, 6, and 8 mmol/liter KCl. Standards were validated against a certified IL calibrator containing 140 mmol/liter Na and 5.0 mmol/liter K (Instrumentation Laboratory, IJsselstein, The Netherlands). Two quality control solutions to estimate the within-assay and between-assay coefficients of variation contained 129 mmol/liter NaCl plus 5 mmol/liter KCl, and 29 mmol/liter NaCl plus 2.5 mmol/liter KCl in the abovementioned solution. The solutions were stored in aliquots at −20°C. As a chemical matrix modifier, a solution containing 1 mol/liter $(NH_4)_2HPO_4$ and 1 mol/liter HNO_3 was used. To investigate whether analyte matrix effects were present, standard addition studies to tubular fluid samples were performed with two matrix modifier solutions which contained additionally 40 mmol/liter NaCl plus 2.0 mmol/liter KCl, and 120 mmol/liter NaCl plus 5.0 mmol/liter KCl.

Equipment

Volumetric constriction pipettes of about 2 nl were made with a microforge as described by Andreucci [5]. Pipetting was performed with a Brinkmann RP-3 micromanipulator (Mannheim, Germany) and an Olympus VMT-45 binocular microscope (Tokyo, Japan). For the measurements, a TJA SH-12 flameless atomic absorption spectrometer was used (Thermo Jarrell Ash Europe, Breda, The Netherlands), provided with Juniper sodium (nr. 4143) and potassium (nr. 4154) hollow cathode lamps (Harlow, Great Britain). The spectrometer was equipped with a TJA CTF-188 controlled temperature graphite furnace with a rectangular graphite cuvette, in which a 11 × 8 mm graphite microboat fits. The settings for the spectrometer

Table 1. Relative sensitivities for sodium and potassium at various wavelengths

Sodium		Potassium	
Wavelength *nm*	Relative sensitivity	Wavelength *nm*	Relative sensitivity
330.2	1	404.2	0.4
589.0	100	766.5	200
589.6	60	769.9	100

Table 2. Furnace temperature settings for determination of sodium and potassium

	Drying	Pyrolysis	Atomization		Cleaning
Temperature *°C*	125	750	2100	1800	2100
Ramp time *seconds*	5	5	0	0	0
Hold time *seconds*	5	5	1	3	2

were: high voltage 900 V, current 5.0 mA, wavelengths 330.2 nm for sodium and 769.9 nm for potassium, slit width 0.3 mm, double beam mode, peak area integration 3.5 seconds with a delay time of 0.5 seconds, and temperature cycle settings as shown in Table 2. Argon was used as the purge gas; the argon flow was stopped during the atomization step. Absorbance was measured in the peak area mode. Background corrections were not required. Contamination by carry-over was not detectable, as established by processing blanks immediately following standards. The SH-12 spectrometer is not equipped to measure two elements simultaneously, but upgrading by the manufacturer to the SH-22 model permits simultaneous measurement of two elements.

Procedure

Tubular fluid samples were obtained from 225 to 275 g male Sprague-Dawley rats on a normal sodium intake. The animals were anesthetized by intraperitoneal injection of 60 mg/kg body weight sodium pentobarbital. The left kidney was exposed by a flank incision and prepared for micropuncture. Throughout the experiments, the rats received an infusion containing 0.9% sodium chloride and 1.0% bovine serum albumin at a rate of 20 μl/min. Late proximal and early distal tubule segments were identified by intratubular injection of small volumes 2% Fast Green in ATF. The presence of Fast Green does not interfere with the determination. Proximal and distal tubular fluid samples were collected for five and ten minutes, respectively, using sharpened glass pipettes with outer diameters of 10 to 13 and 8 to 10 μm, filled with water-saturated mineral oil. The samples, ranging from 60 to 150 nl, were stored in the micropuncture pipettes at −20°C. Determinations were performed within two weeks after collection. To prevent evaporization by exposure to air, micropuncture samples were transfered with the micromanipulator into a siliconized petri dish containing water-saturated light mineral oil. Standard, quality control, and matrix modifier solutions were also pipetted into the mineral oil. With the 2 nl constriction pipette and the micromanipulator, one volume matrix modifier and one volume tubular fluid, separated by an oil block, were transfered to the surface of the graphite microboat. All determinations were performed in triplicate. After each sample, the pipette was cleaned with acetone, chromic acid and ultrapure water.

Results

As shown in Figure 1, standard lines of sodium and potassium absorbance against concentration, both in UPW and ATF, are linear ($P < 0.001$). The standard lines in UPW and ATF did not differ significantly from each other by analysis of covariance ($P > 0.05$). The sensitivity, expressed as the amount of electrolyte yielding an absorbance of 0.0044, is 6 pmol for Na, and 0.06 pmol for K, resulting in limits of detection of 3.0 and 0.03 mmol/liter, respectively. In Table 3, the within-assay and between-assay coefficients of variation are given. Table 4 shows that sodium and potassium added to tubular fluid samples were completely recovered. Reference values (± SD) for late proximal ($N = 36$) and early distal ($N = 30$) tubular fluid samples of male Sprague-Dawley rats are 140 ± 5 and 48 ± 9 mmol/liter for Na, and 4.72 ± 0.52 and 1.73 ± 0.50 mmol/liter for K, respectively.

Discussion

The currently presented method for the measurement of sodium and potassium in tubular fluid samples has two advantages compared to other published atomic absorption spectrometry methods [1–4]. At the commonly used wavelength of 589.0 nm, the amounts of sodium originating from dust and tubular fluid are comparable of magnitude. To eliminate the dust problem, the analytical equipment must be placed in a dust-free room [2]. As an alternative, tubular fluid samples have been diluted with water and measured manually at 589.6 nm [3], or by autosampler at 330.2 nm [4]. Since the degree of dilution is large, contamination of water with only traces of sodium gives a huge increase in background absorption and a concomitantly reduced signal-to-noise ratio [3]. As proposed in the present method, sacrificing some sensitivity by analysis at a less sensitive wavelenght makes the relative contribution of sodium from dust to the measured absorbance negligible. The amount of sample needed for determination (3 × 2 nl) is still markedly lower than the amount needed for an autosampler (200 nl), and it is small compared to the total amount generally collected by us in micropuncture studies (60 to 150 nl). The remainder of the sample is sufficient for determination of other constituents, for example, lithium (3 × 10 nl) [6] or inulin (3 × 8 nl) [7]. The reduction in sensitivity does not invalidate measurement in distal tubular fluid samples, since the detection limit of sodium is at least 15 times lower than the expected concentrations.

By reducing the sensitivity for analysis of potassium as well, but to a considerably less extent than for sodium, the elements can be analyzed in the same absorbance range despite the large differences in concentration. Thus, higher sodium concentrations as present in proximal tubular fluid can be measured in the linear absorbance range. At higher sensitivities, this is impossible even with the smallest practicable constriction pipettes [2], whereas dilution of the sample results in loss of sensitivity for potassium [3]. This dilemma is avoided by selecting wavelengths with a potassium-to-sodium sensitivity ratio in the same

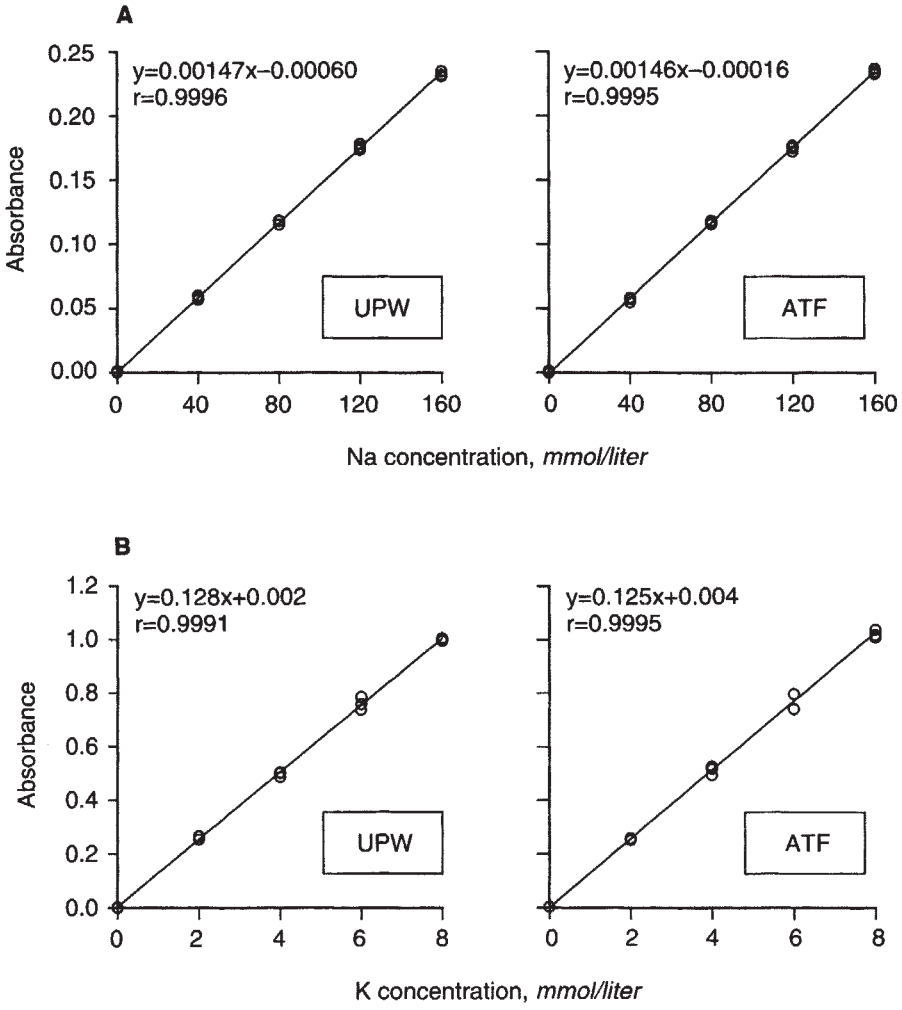

Fig. 1. *Standard lines of absorption plotted against concentration of sodium (A) and potassium (B), both made in ultrapure water (UPW) and artificial tubular fluid (ATF).*

Table 3. Within-assay and between-assays coefficients of variation

	N	Na, 40 mM	Na, 140 mM	K, 2.5 mM	K, 5.0 mM
Within-assay %	12	2.8	1.5	4.5	2.6
Between-assays %	12	4.8	2.6	6.3	4.8

Abbreviations are: Na, sodium; K, potassium; mM, mmol/liter.

Table 4. Recovery of standards added to tubular fluid (± SD)

	Endogenous concentration	Na added: 40 mM K added: 2.0 mM	Na added: 120 mM K added: 5.0 mM
Na	42 mM	102 ± 4%	99 ± 3%
K	2.1 mM	103 ± 5%	102 ± 2%
Na	135 mM	98 ± 6%	ND
K	4.8 mM	102 ± 4%	ND

Abbreviations are in Table 3; ND, not determined.

range as the sodium-to-potassium concentration ratio. At the currently proposed wavelengths, the sensitivity ratio increases from 2 to 100, which is comparable in magnitude to the average concentration ratio of 30.

The findings that standard lines made in ultrapure water and artificial tubular fluid are identical, and that standards added to tubular fluid samples are recovered completely, indicate that interfering effects from other solutes are absent. In sum, the proposed method for nanoliter analysis of sodium and potassium in tubular fluid is reliable, both elements can be analyzed simultaneously in the same absorbance range, and interference by sodium from contaminations is eliminated.

Acknowledgement

This study was supported by Pioneer Grant 900-795-233 of the Netherlands Organization for Scientific Research N.W.O.

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